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Scientific Note

EFFECTS OF COPULATION FREQUENCY ON EGG-LAYING AND EGG HATCH IN THE WALNUT HUSK FLY, *RHAGOLETIS COMPLETA* CRESSON

The mating system of *Rhagoletis completa* Cresson (WHF) has been characterized as a resource-based polygamous system (Opp, S. B. et al. 1996. In: Steck, G. J. and B. A. McPheron (eds.), Fruit Fly Pests: A World Assessment of Their Biology and Management. St. Lucie Press, Fla.), in which males actively defend walnuts to gain exclusive mating opportunities with females seeking oviposition sites. Post-copulatory mate guarding and multiple matings by males and females have been observed in field studies (Opp, et al. 1996) but nothing is known about the effects, negative or positive, of multiple mating behavior on female fertility and fecundity. This study determined the effects of copulation frequency (single vs. multiple with each of two mates) on WHF egg-laying propensity and egg hatch in the laboratory.

WHF adults developed from larvae that were collected from infested walnuts from Ardenwood Historic Farm (East Bay Regional Parks, Newark, Calif.). The larvae pupariated, overwintered, and adults eclosed in the lab. Flies were separated by sex at eclosion to insure virginity and were held at least two weeks under laboratory temperatures of 24 ± 2° C to insure that both sexes had reached reproductive maturity (Boyce, A. M. 1934. Hilgardia, 8: 363–579) prior to use in mating experiments. Adult flies were fed a mixture of sugar and hydrolyzed yeast, and given water.

Matings were conducted in the lab by placing virgin males and females in a 25 cm^3 Plexiglas and screen communal cage which was observed continuously during the mating trial for any copulating pairs. Copulating pairs were carefully removed in copula from the communal cage using a small, clear plastic cup and then were held separately in 470 ml plastic cages where they were supplied with food and water. All initial copulations were timed and only pairs which remained in copula for at least 5 min were used in the trials (average copulation duration = 9 min in this study and previous field studies (Opp, et al. 1996). During a particular mating session, we separated the total number of copulating pairs equally into two mating treatments (single copulation vs. multiple copulations with each of two mates). The single copulation treatment consisted of a male and female pair allowed to copulate once, and, after the pair disengaged, the male was removed from the cage and the female was allowed to oviposit for a period of one week. After one week, a mated female was placed in another container with five virgin males and continually observed for any new matings. The second male was also removed after the pair naturally disengaged, and the female was again allowed to oviposit freely for several weeks. Thus, the single copulation treatment consisted of female flies which had copulated once with each of two males. In the multiple copulation treatment, the male was not removed after the first copulation but rather was transferred along with the female to a cage where

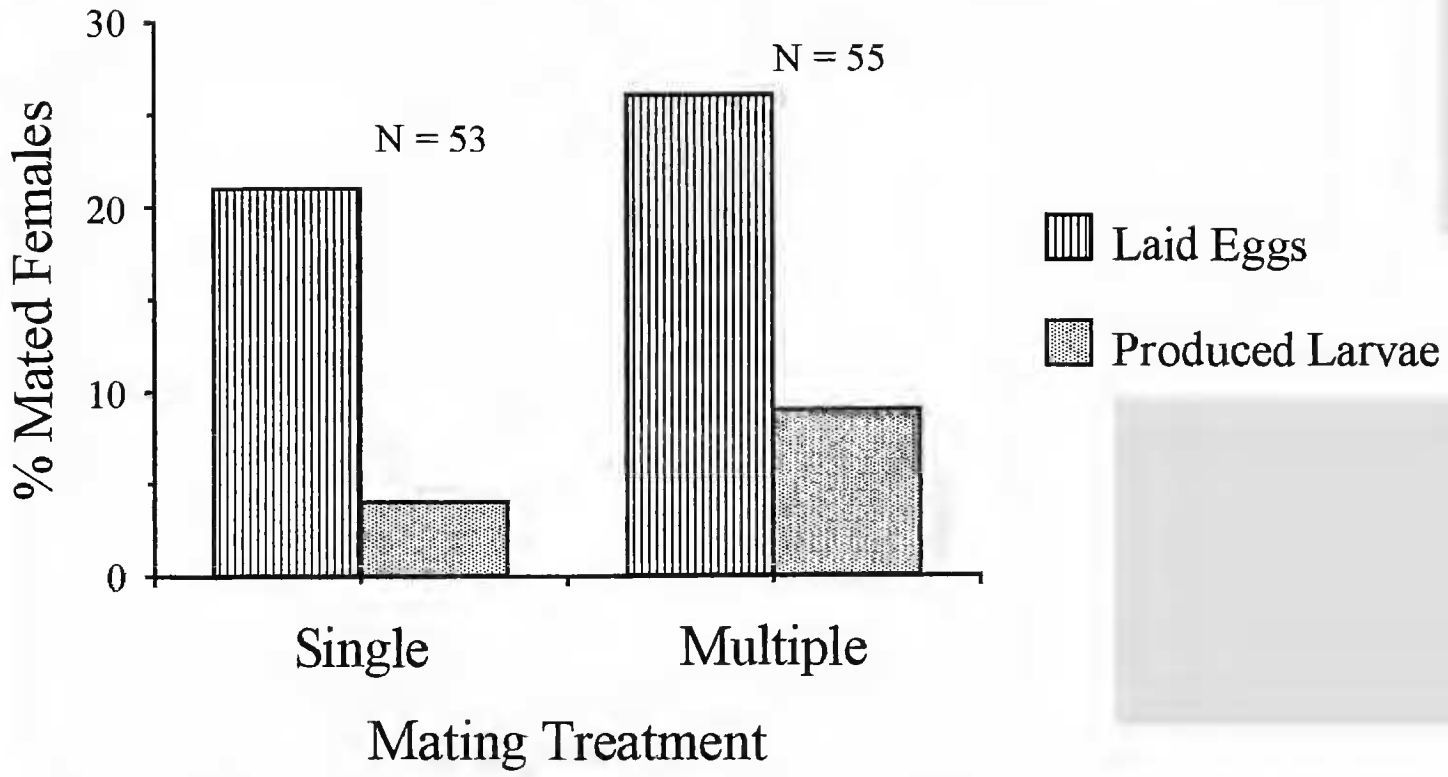

Figure 1. Effect of numbers of matings on egg-laying and larval hatch in the walnut husk fly. (N = number of females in each mating treatment.)

they remained together for 48 h with food and water and an oviposition substrate. The pair was allowed to copulate at will, however the exact duration and frequency of subsequent copulations were not recorded. Most pairs were observed to mate frequently although pairs were not observed continuously beyond the first copulation. After 48 h, the male was removed and the female was allowed to continue ovipositing for the remainder of the week. The following week she was placed in the company of five new virgin males and observed for any matings. A resulting pair was again removed and transferred to a separate cage and allowed to copulate at will for 48 h. The second male was also removed after 48 h, and the female was allowed to oviposit freely for several weeks. Thus, the multiple copulation treatment consisted of females allowed to copulate many times with each of two males.

Females in both treatments received artificial substrates which mimicked the green walnut husks and allowed easy penetration for oviposition (Telang, A. 1995. M.S. Thesis). The artificial walnuts were constructed of 1.8% agar in distilled water colored with green food dye which was poured and allowed to set in plastic snap-together Easter egg molds before being covered with a thin layer of Parafilm®. Fly eggs were collected intact from the agar walnuts and placed on black moistened construction paper stacked on top of layers of moistened filter paper and kept in petri dishes in the dark. Petri dishes were checked daily and egg hatch recorded. Mean numbers of eggs laid per female and mean numbers of hatching larvae per female were compared between treatments with Mann-Whitney U-tests (Sokal & Rohlf. 1981. Biometry. Freeman and Co.). Propensities of females of each mating treatment to lay eggs and to produce fertile eggs were compared with G-tests of independence (Sokal & Rohlf. 1981).

Singly-copulated females were both less likely to lay eggs and less likely to produce larvae than multiply-copulated females (Fig. 1) though these differences were not statistically significant ($G = 0.36$, $df = 1$, $P > 0.05$). Among females

laying eggs, multiply-copulated females laid more eggs (i.e., showed greater fecundity) than singly-copulated females (multiply-copulated = 49.1 eggs/female; singly-copulated = 17.2 eggs/female) even though these differences were not statistically significant (W = 126.5, $P = 0.38$). Multiply-copulated females also demonstrated greater fertility than singly-copulated females (multiply-copulated = 37.2 hatching eggs/female; singly-copulated = 5.0 hatching eggs/female) though, again, no statistically significant difference was detected (W = 5.0, $P = 0.33$).

We concluded that females allowed multiple copulations had higher levels of egg-laying and egg hatch than females only allowed a single copulation with each mate. The lack of statistically significant differences likely resulted from low sample sizes and high variability but does not detract from the biological significance of these findings. Repeated copulations may grant females a nutritional, stimulatory or sperm replenishment benefit as reported for a congener, *R. pomonella* (Walsh) (Opp & Prokopy. 1986. Ann. Entomol. Soc. Amer., 79: 705–710). Our study, however, was not designed to test any of the above mentioned hypothetical benefits of multiple copulations in the WHF but to determine the optimal mating conditions for collection of WHF offspring for laboratory paternity analysis. Based on our study, we recommend that WHF females be allowed to mate multiply (i.e., at least twice) with each mate to increase laboratory offspring production. These results correlate well with field studies which have reported that female WHF on average mate approximately twice a day, often with the same male, and may mate as many as nine times a day with one or more males (Opp et al. 1996).

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